Pregnancy—A Critical Time for Mental Health

Interrogating Psychiatry with Microbiota-Gut-Brain Axis and Autonomic Nervous System Biomarkers

MARY C. KIMMEL
Abstract

Perinatal mood and anxiety disorders (PMADs) are common and impact the parent and child beyond the perinatal period of pregnancy and postpartum. The aim of this thesis was to study biomarkers that might reflect perinatal mental health. This work focused on multiple methods of mental health characterization and the autonomic nervous system as reflected by heart rate variability (HRV), the immune system, and the gut microbiome.

Three observational longitudinal cohorts of pregnant individuals from three geographic regions were studied: 1) the Biology, Affect, Stress, Imaging and Cognition (BASIC) and the follow-up study U-BIRTH from Uppsala University Hospital; 2) the University of Illinois at Chicago (UIC) MoMent cohort; and 3) the University of North Carolina at Chapel Hill (UNC) cohort.

The first paper assessed HRV before and after a mental task and in relation to psychiatric diagnoses, exposure to trauma, and self-report of mental distress. The second paper studied the Perceived Stress Scale (PSS-10) in relation to microbial composition and T-cell related cytokines and chemokines. The third paper studied the Edinburgh Postnatal Depression Scale (EPDS) in relation to whole genome sequencing and Gut Brain Modules for functioning. The fourth paper assessed trajectories of infant temperament in relation to depression and anxiety from the EPDS.

The PSS-10 and the EPDS factored differently in the cohorts. HRV patterns differed based on anxiety disorder type, greater trait anxiety, and greater exposure to childhood traumatic events; microbiome data improved the prediction of PSS-10 self-efficacy; and self-efficacy was associated with a bacteria type more beneficial in the presence of dietary fiber that also associated with an immune factor important in immune tolerance. Greatest variation in microbial community functioning was due to cortisol degradation and synthesis of inositol, menaquinone, and the short chain fatty acid (SCFA) acetate. Anxiety in pregnancy was associated with children who had higher levels of sensitivity and greater negative affectivity that increased over early life.

These four papers highlight: 1) the course of mental health in pregnancy is critical to the development of parent and child; 2) the characterization of perinatal mental health requires a mix of methods that recognize there may be differences in the use of the methods based on the population; and 3) biomarkers of perinatal mental health need to reflect dynamic systems, and the components may not be as important as the patterns and interactions.

Keywords: pregnancy, heart rate variability (HRV), microbiome, depression, anxiety, self-efficacy, cytokines, biomarkers

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ISSN 1651-6206
URN urn:nbn:se:uu:diva-512959 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-512959)
Shared joy is double joy; shared sorrow is half a sorrow—
Swedish Proverb
To my boys, my parents and brother, my mentors, my NC MATTERS teammates, my community, and my patients
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


IV. Sörensen F., **Kimmel M.C.**, Brenner V., Krägeloh-Mann I., Skalkidou A., Mahjani B., Fransson E. Interactions of perinatal depression versus anxiety and infants’ early temperament trajectories. (provisionally accepted to *Child Development*).

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Abbreviations

ANS Autonomic Nervous System
ASV Amplicon Sequence Variant
BASIC Biology, Affect, Stress, Imaging and Cognition
BMI Body Mass Index
CXCL C-X-C Ligand
DNA Deoxyribonucleic Acid
DSM Diagnostic and Statistical Manual
DSRS Depression Self-Rating Scale
ECBQ Early Childhood Behavior Questionnaire
EPDS Edinburgh Postnatal Depression Scale
GAD Generalized Anxiety Disorders
GBM Gut-Brain Modules
FDR False Discovery Rate
HPA Hypothalamic-Pituitary-Adrenal Axis
HRV Heart Rate Variability
    HF High Frequency
    LF Low Frequency
    RMSSD Root Mean Square of Successive Differences
    SDNN Standard Deviation of the Interbeat Intervals
    VLF Very Low Frequency
IBS Irritable Bowel Syndrome
ICQ Infant Characteristics Questionnaire
I-TAC Interferon-inducible T Cell Alpha Chemoattractant
LITE Lifetime Incidence of Traumatic Events
LMM Linear Mixed Model
LPS Lipopolysaccharide
MINI Mini-International Neuropsychiatric Interview
NARSAD National Alliance for Research on Schizophrenia & Depression
NIMH National Institutes of Mental Health
PMAD Perinatal Mood and Anxiety Disorder
PPD Postpartum Depression
PSS Perceived Stress Scale
    ED Emotional Distress/rED reduced Emotional Distress
    PS Perceived Stress/rPS reduced Perceived Stress
    SE Self-Efficacy/rSE reduced Self-Efficacy
Introduction

After experiencing three first trimester losses and then several years without being able to become pregnant, a patient became pregnant with twins following in vitro fertilization and carried the pregnancy through first trimester and second trimester. At 28 weeks the patient’s anxiety increased and was referred to meet with the mental health team. Patient reported an impending sense of doom and sudden onset of panic where she was sure she was dying. Patient had had a similar episode in college. In the meeting with the psychiatry team, she had an elevated blood pressure, followed by two normal blood pressures; but still higher for the patient than usual. Records indicated no lab anomalies from the prior obstetric visit earlier that week where one elevated blood pressure was also noted, and also followed by blood pressures that were in the normal range. Psychiatry encouraged close follow-up with obstetrics. Patient’s own medical history, in addition to unexplained infertility, included irritable bowel syndrome (IBS) and a period where she had exercise-induced anaphylaxis in college. The latter was treated with steroids and antihistamines. While the patient has not been treated for anxiety or depression in the past, she has a strong family history of mood and anxiety disorders, including a brother having had severe panic disorder with agoraphobia and died by suicide and the patient’s mother having been hospitalized multiple times for manic and depressive episodes. The patient had been screened at the first prenatal visit with the Edinburgh Postnatal Depression Scale (EPDS)(Cox et al., 1987), and had scored nine out of thirty; patient had declined mental health support at time.

The day after the psychiatry visit, the patient was not feeling well, and remembering the conversation with psychiatry, the patient went to labor and delivery. Patient was found to have blood pressures of 180s/100s and was admitted. One of the babies was found to be small for gestational age and the biophysical profile was not reassuring. The patient was delivered by c-section. Patient learned after the c-section that her mother had also had preeclampsia.

When patient presented for the six-week postpartum visit, the patient said that despite the babies progressing well per the Neonatal Intensive Care team, the patient had had significant distress and kept feeling at fault for the prior losses and for what the babies were going through. The patient admitted to beginning to think the children would be better off with a different parent. The patient’s partner said the patient had talked about being a broken person and
convincing the rest of life would be in and out of psychiatric hospitals like her mother and then die by suicide like her brother. Patient now admitted having some of these thoughts starting in the early second trimester. Patient had not told the obstetric team for fear of judgement; and, at first, had been able to push the thoughts away with usual tools such as going for walks, immersing in work, and talking with good friends. She no longer felt motivated for or interested in these activities. This was the first time she had thoughts about suicide. Staff note scratches on her wrist; and she said she was trying to see how it felt to use a knife on her wrist. The patient is unsure about treatment with medication having seen her family members have poor responses to medication. Patient says others tell her medication in the breastmilk would negatively impact her children. The patient notes pumping breastmilk is the only thing done that feels like good parenting.

The above story is an amalgamation of patients for whom I have provided care; patients and their stories that have driven a desire to improve assessment and treatment of mental health. Mental health is often thought of as the absence of a mental health disorder. Mental health disorders are defined by the Diagnostic and Statistical Manual of Mental Disorders or the International Classification of Diseases (American Psychiatric Association, 2000, 2013). Mental health disorders have been an attempt to group together symptoms into diagnoses that might explain groups of symptoms. Occam’s Razor, first presented to me in medical school, suggests that we should look for simpler explanations such as the patient’s multiple co-morbidities including panic, immune-related reactions, and bowel distress might be connected by a similar biologic process (Hilliard et al., 2004; J. Kelly, 2021).

However, the U.S. Department of Health and Human Services defines mental health as not only biology, but life events and family history (U.S. Dept of Health and Human Services, n.d.). Family history may both reflect biology such as genetics, but also shared experiences. Pregnancy is a period of not only rapid biologic adaptation but also a major life event with psychologic components; the process of the development as a parent. The case highlights how worries about judgement and social factors can impact how a patient will disclose symptoms and how they themselves see their symptoms. The patient’s story reflects pregnancy as dynamic and interactive. Throughout pregnancy the pregnant person is reacting and adapting to the developing child; just as the developing child is reacting and adapting to the environment of the parent and the reactions of the parent, forming the basis for preparing the developing child for the future (Martini et al., 2017); in fact, the pregnant person, the non-birth parent, their main supports, and the offspring are all a complex network that is adapting, integrating, and developing (Garthus-Niegel et al., 2021).

The aim of my research is to study systems that might reflect and integrate measures of physiologic components with interactive environmental processes and the person’s characterization of their mental health. My training in
obstetrics and psychiatry have provided me a foundation in a medical model of diagnosis and treatment of mental disorders. And yet my work with patients, such as the patient described, has increasingly implicated the need for learning and implementing systematic science in order to better understand and support perinatal mental health.
Background

Perinatal Mood and Anxiety Disorders

One in four pregnant individuals have been found to have elevated depressive symptoms in the third trimester (Okagbue et al., 2019). One in five pregnant individuals meet criteria for an anxiety disorder during pregnancy (Fawcett et al., 2019). One in ten pregnant individuals have reported thoughts of self-harm (Kalmbach et al., 2020). The term perinatal mood and anxiety disorders (PMADs) has increasingly been used by many individuals because it acknowledges the range of presentations, both in timing and in type of symptoms (M. M. Long et al., 2019). However, the use of the term PMADs has also come under scrutiny because of the acronym results in the word “mad” (Hutchens & Likis, 2019). Characterization of mental health during the perinatal period, i.e., pregnancy and the postpartum period, has been challenging because of the diversity of presentations and the dynamic nature of pregnancy.

Perinatal mental health is most often been traditionally assessed by the Edinburgh Postnatal Depression Scale (EPDS) (Cox et al., 1987), which is not only a measure of depressive symptoms but also anhedonia and anxiety symptoms (K. T. Putnam et al., 2017). The EPDS was designed to acknowledge the common occurrence of anxiety symptoms in the perinatal period by inclusion of three questions related to anxiety; and also have been validated in pregnancy despite its name with regards to postnatal (Heller et al., 2022; Matthey, 2008). The EPDS is an important tool utilized world-wide; however, the EPDS is limited to 10 questions and therefore cannot capture all the mental distress symptoms that occur in pregnancy including symptoms that may be most distressing and impairing to some individuals. Individuals may not feel it captures their experiences (e.g., feeling they are anxious as opposed to “for no good reason”). The EPDS is limited in assessing other symptoms common to the perinatal period such as the obsessive compulsive symptoms, intrusive thoughts, post-traumatic stress, and mania (Abramowitz et al., 2010; Yildiz et al., 2017). The language may be interpreted differently depending on the cultural context; and an individual may answer differently depending on the setting in which the patient is being given the screener. Furthermore, individuals may worry about judgement if answering positively about symptoms of distress. In a survey of 1,500 women, over 70% admitted to minimizing or completely hiding their symptoms when answering questions on the EPDS (Russell et al., 2013).
Perceived Stress

Half of third trimester individuals met criteria for moderate perceived stress and additional 5% with severe perceived stress (E. S. Long et al., 2023). Higher perceived stress in pregnancy is associated with greater risk of major depression and anxiety (Aas et al., 2020; Biaggi et al., 2016; Woods et al., 2010). Higher perceived stress is also associated with preterm birth, preeclampsia and other obstetric complications (Gerson et al., 2021; Monk et al., 2020). However, it important to note that not all stress is negative. The March of Dimes website writes “High levels of stress that continue for a long time may cause health problems, like high blood pressure and heart disease…Stress is not all bad. When you handle it right, a little stress can help you take on new challenges”(March of Dimes, n.d.).

Perceived stress as defined by Cohen’s Perceived Stress Scale: succumbing to nervousness, fear, and anger and perceiving that one’s life is out of control with troubles too great to surmount. The most common measure of perceived stress is the Perceived Stress Scale (PSS)-10 which can be separated into two factors (perceived distress and perceived self-efficacy) (Cohen et al., 1983; Cohen & Williamson, 1988; Taylor, 2015). These two factors reflect both distress contributing to perceived stress, balanced with sense of self-efficacy.

Biomarkers

Biomarkers have been defined as a “distinct biochemical, genetic, or molecular characteristic that is an indicator of a particular biological condition or process” (“Biomarkers and Surrogate Endpoints,” 2001; Kirkpatrick et al., 2021). Results for psychiatric biomarkers may initially seem promising and then are not replicated (Kirkpatrick et al., 2021). Longitudinal and larger studies have the potential to improve identification, especially in evolving biology such as in pregnancy; but also must ensure accounting for heterogeneity in diagnostic practice, co-morbidities, and biases in subject recruitment (Abi-Dargham et al., 2023; Kirkpatrick et al., 2021). Mental health characterization beyond diagnoses and a systems biology approach are needed (Abi-Dargham et al., 2023).

Heart Rate Variability (HRV) Reflects Autonomic Nervous System (ANS) Health

Patient stories about their mental health often include descriptions of physical symptoms such as heart racing. Heart rate variability (HRV) is thought to reflect different components of the autonomic nervous system (ANS); with greater variability connoting increased adaptability and improved health (e.g., lower risk of cardiac mortality, anxiety and depressive disorders, and posttraumatic stress disorder (Beauchaine & Thayer, 2015; Shaffer & Ginsberg, 2017;
HRV can be characterized by average and standard deviation of the length between beats or by the frequencies that make up the heart rate oscillations; these measures are thought to reflect different aspects of the ANS such as the Root Mean Square of Successive Differences between heart beats (RMSSD) and the high frequency measure of HRV (HF) reflecting vagal activity inputs (Shaffer & Ginsberg, 2017), while the low frequency band (LF) is thought to reflect sympathetic activity along with the baroreflex important in maintaining balance between blood pressure and heart rate, and the very low frequency (VLF) is thought to reflect some components of the renin-angiotensin system along with aspects of the sympathetic activity (Ernst, 2017; Shaffer & Ginsberg, 2017). The standard deviation of the IBI of normal sinus beats (SDNN) is thought to reflect risk of cardiac morbidity and mortality; with a SDNN below 50 as indicating higher risk, while a SDNN above 100 is considered a marker of health (Electrophysiology, 1996; Kleiger et al., 1987; Shaffer & Ginsberg, 2017). HRV measures are also thought to encompass changes resulting from inflammation (Ernst, 2017; Haensel et al., 2008; Williams et al., 2019).

HRV may be able to reflect how an individual is navigating changes. However, research is limited with regards to the breadth of mental health characterizations, and in particular, with regards to PMADs (Abbas et al., 2005; Braeken et al., 2015; DiPietro et al., 2005; Ekholm & Erkkola, 1996; Fu, 2018; Gandhi et al., 2014; Kuo et al., 2000; Logan & Yeo, 2017; Maser et al., 2014; Mizuno et al., 2017; Stein et al., 1999).

Inflammation and Perinatal Mental Health

Pregnancy requires significant adaptation of the immune system (Osborne & Monk, 2013). A pro-inflammatory state occurs with implantation; then followed by immune tolerance of the fetus balanced with immune activation when necessary from the second trimester into the thirds, and then increase of inflammation again to prepare for delivery (Osborne & Monk, 2013; Ravi et al., 2022).

Bacterial toxin, lipopolysaccharide (LPS), has been found to induce negative mood and anxiety in non-pregnant adults in addition to classic physical signs of inflammation (Lasselin et al., 2020). In a study of giving LPS, cortisol response was blunted in obese individuals compared to non-obese individuals and thought to be due to chronic low grade inflammation and elevated cortisol; possibly providing some insight into how a pregnant individual might similarly navigate an inflammatory trigger given the loss of negative feedback by cortisol on the hypothalamic-pituitary-adrenal axis in order to have higher amounts of cortisol to support the developing fetus (Lasselin et al., 2020). The immune system factors associated with pregnancy and mood and anxiety have be highly varied in findings (Bränn et al., 2017, 2019; Edvinsson et al., 2017; Osborne et al., 2019). In another study of non-pregnant adults, half of
individuals met criteria for a DSM-V depressive or anxiety disorder and these individuals had higher LPS in the blood and higher levels of zonulin and fatty acid-binding protein-2, markers of intestinal permeability and associated with upregulation in presence of more pathogenic gut bacteria (Stevens et al., 2018). This provides support for inclusion of the microbiome in better understanding the adaptation of the immune system in pregnancy, microbiome both as trigger and reflective of the host immune system, and reflecting when inflammation is excessive.

The Microbiome and the Microbiota-Gut-Brain Axis

Within the gastrointestinal tract live a community of microbes. The word microbiome originates from the three parts: “micro” meaning small, “bio” meaning life, and “ome” meaning a whole; such that the microbiome is an ecological community that is a “reasonably well-defined habitat which has distinct physio-chemical properties” (Berg et al., 2020; Lederberg & Alexa T. McCray, 2001; Whipps et al., 1988). The microbiome may include different types of microbes, the microbiota, and their 10 million distinct microbial genes (Berg et al., 2020; Li et al., 2014); and is specific to the individual and reflects one’s diet, lifestyle, medications, infections, and adversity (Zeng et al., 2017).

Improved gene sequencing methods have led to the ability to better characterize the microbiota and their functions (Caporaso et al., 2010; Gilbert et al., 2018; Jovel et al., 2016). Characterization of the microbiome includes more global measures of each person’s community: alpha diversity characterizes the number of different types of microbes, the evenness of relative abundance of microbe types, and the genetic similarity of the microbes; beta-diversity characterizes based on genetic similarity and relative abundance how different is one individual’s community from other individuals grouped by a factor such as high or low mental distress (Bastiaanssen et al., 2021; Walters & Martiny, 2020). New analytical tools such as building networks increases our ability to see how these different components interact (Peñalver Bernabé et al., 2018).

The microbiota-gut-brain axis has emerged as a reflection of the host-environment interface. The microbiota-gut-brain axis includes: a) the microbes; b) the communicators that take messages from the microbes to the brain and from brain to the microbes (e.g., metabolites created by the microbes, host metabolites, host immune system factors, the nerves such as the vagus nerve); and c) the brain that interprets the messages and then reacts (Codagnone et al., 2018; Cryan & Dinan, 2012; Foster et al., 2017; Fülling et al., 2019; Gilbert et al., 2018). The microbiome has been found to be important in development of a number of host systems such as the host immune system and the Hypothalamic-Pituitary-Adrenal Axis (Gur et al., 2017; Sudo et al., 2004). The microbiota-gut-brain axis, outside of pregnancy, has been associated with
psychiatric disorders including Major Depressive Disorder and Generalized Anxiety Disorder (Y. Huang et al., 2018, 2018; H. Jiang et al., 2015; H.-Y. Jiang et al., 2018; J. R. Kelly et al., 2016).

The perinatal period, is a “natural stress test,” even in healthy pregnancies, which may alter the maternal intestinal microbiome; and is also important to the development of child’s microbiome (Bilhartz et al., 2011; Gur et al., 2017; Sudo et al., 2004; Virani et al., 2021). The largest cohort, to date, of pregnant individuals (n=1,479) determined that the microbiome in pregnancy is dependent on factors such as age, weight gain, and the presence of pre-pregnancy and gestational diseases such as hypertension (Yang et al., 2020). The maternal microbiome has been implicated in preterm birth, preeclampsia, and gestational diabetes (Kuang et al., 2017; Lv et al., 2019; Vinturache et al., 2016).

Furthermore, the maternal microbiome may impact offspring development. A less diverse microbiome in the third trimester of pregnancy was associated with greater child internalizing behaviors at 2 years; normative childhood behavior associated with higher amounts to butyrate-producing taxa (Dawson et al., 2021).

Maternal Stress and Infant Outcomes

Maternal stress is not only associated with negative outcomes for the pregnant person, but also for the offspring. Maternal stress is associated with behavioral, motor, and cognitive problems, increased risk of psychopathology later in life, and differences in central nervous system and ANS development (Van Den Bergh et al., 2020).

Transgenerational transmission of stress which may be reflected in alterations in the child’s reactivity, self-regulation, and interaction with the environment and other individuals. Even soon after birth, infants have different behavioral styles that reflect how they may differentially interact with environment and self-regulate; these behaviors can include vocalizations, crying or fussing, and facial reactions (Mayberry & Affonso, 1993; Medoff-Cooper, 1995). Infant temperament, the characterization of these different behavioral styles, has been associated with implications for behaviors and illnesses later in life (Riese, 1987). Temperament, that includes a tendency to react more strongly, is associated with later development of psychiatric disorders (Rettew & McKee, 2005). An infant with a “difficult” temperament, more sensitive to stressors and struggles with adaptation and change, is more likely to develop psychiatric disorders (Austin et al., 2005; Boyce & Ellis, 2005; Brannigan et al., 2020).

Research of the impact of maternal depression on infant temperament development has shown variable results. Depression in pregnancy may have independent effects of postpartum depression on infant temperament (Fransson et al., 2020).
Aims

The overall aim of this thesis is to study the associations of varied methods of characterizing perinatal mental health with biomarkers reflecting the ANS and microbiota-gut-brain axis.

I. To analyze HRV profiles during late pregnancy before and after a mental task in relation to psychiatric diagnoses and mental health factors

II. To determine how perceived stress and its components of distress and self-efficacy during pregnancy in two different USA cohorts (one suburban, highly-resourced and the other urban, lower resourced) are associated with microbial composition and immune factors

III. To utilize both a USA cohort and a Swedish cohort, to explore self-reported mental distress measures in relation to microbial composition and microbial potential functioning

IV. To identify distinct trajectories of difficult infant temperament, as reported by the mother over the first 18 months after childbirth; and to assess trajectories in relation to maternal symptoms of depression and anxiety in pregnancy and postpartum
Methods

Design
The studies in this doctoral thesis are performed with three observational cohorts of pregnant individuals from three different geographic regions with longitudinal design and repeated measures: 1) the Biology, Affect, Stress, Imaging and Cognition (BASIC), a population-based study at Uppsala University Hospital, Sweden of pregnant individuals recruited at 17 weeks and followed through 12 months postpartum with web surveys; and include a subset invited for research laboratory visits at gestational week 38 (Axfors et al., 2019); 2) pregnant individuals recruited by the University of Illinois at Chicago (UIC) before 16 weeks and followed in one timepoint in the third trimester (Wenzel et al., 2022, 2022); and 3) pregnant individuals recruited at the University of North Carolina at Chapel Hill (UNC) in the first or second trimester of pregnancy and followed again in the third trimester and postpartum; with subset of women’s infants at 2 months postpartum (E. S. Long et al., 2023).

For the two papers with data from two cohorts, measures characterizing mental health were reviewed from each cohort in order to identify shared measures. All three studies collected fecal samples for microbiome analysis. Two cohorts measured HRV before and after a mental stressor. Tables I-III list mental health assessment, other covariate assessments, and biomarkers included in each paper. Papers I, III, and IV include data from the BASIC (“Biology, Affect, Stress, Imaging and Cognition in pregnancy and puerperium”) cohort from Uppsala University. Paper II and III included data from the USA UNC cohort; developed through a USA National Institute of Mental Health (NIMH) K23 training grant and funding from a NARSAD Young Investigator Award from the Brain and Behavior Research Foundation. Paper II includes data from MoMent from UIC in the USA. Paper IV utilized the follow-up study to BASIC, U-BIRTH.

Ethics
The BASIC project, as well as and the U-BIRTH project on children follow-up, have been approved by the Regional Ethical Review Board in Uppsala, Sweden (Dnr 2009/171, with amendments 2016/4). The studies followed the ethical guidelines set out by the Swedish Ethical Review Authority and GDPR.
requirements and were conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants to participate in the BASIC and U-BIRTH.

Study protocols for the two USA cohorts were approved by the respective organizational Institutional Review Boards: UIC with IRB# 2014-0325 and IRB# 2018-0842; and UNC with IRB# 16-0959 and IRB# 16-2783. All individuals were consented before engaging in the studies at the respective sites.

Settings

Uppsala, Sweden
The Biology, Affect, Stress, Imaging and Cognition (BASIC) cohort was recruited through Uppsala University (Axfors et al., 2019). BASIC was started in 2009 to gather data and biomarkers from pregnant individuals followed through the postpartum period. Women were recruited during their routine ultrasound visit at 17 weeks. Exclusion criteria included <18 years, unable to speak fluent Swedish, protected identity, blood borne infectious diseases, and non-viable pregnancies. Participants were given web surveys to complete. A subset of the participants was invited to a laboratory visit occurring in the study research space. When a participant’s child reached 18 months, participants from BASIC were offered inclusion in the U-BIRTH study; and if they consented and were willing to fill out additional questionnaires about the child’s development and their own wellbeing, they were included.

Chapel Hill, North Carolina (NC), USA
The cohort of subjects recruited from the UNC at Chapel Hill obstetric clinics, campus advertising, and a local birth center. Primary form of recruitment was flyers and participants then reached out to the study team if interested. This cohort was funded by a K23 training grant from the USA NIMH and a Brain & Behavior Research Foundation NARSAD Young Investigator’s award. Participants were given a web-survey associated with each visit. All visits occurred in a behavioral laboratory on campus.

Chicago, Illinois (IL), USA
The subjects from UIC are part of the larger cohort named MoMent: A Research Study on Moms & Mental Health. Pregnant individuals were recruited from their obstetric visits before 16-week gestation at the UIC. All visits were done in clinic during obstetric visits.
Study Populations

Paper I

Between January 2010 and December 2018, 715 were invited with 349 laboratory visits completed. A greater number invited of the larger BASIC cohort for the laboratory visit had higher numbers with elevated EPDS scores (half of the participants had elevated EPDS score above 12 in the 32 week web survey) (Axfors et al., 2019). Heart Rate Variability (HRV) was added later in the study to the 38-week laboratory visit; resulting in 126 participants with HRV data.

HRV was also available for a non-pregnant comparison group that were aged 22-42 years of age, BMI within range of 20-29 kg/m², with parity less than 4, no systemic disease or current psychiatric condition. The non-pregnant comparison group had not been pregnant during the past two years and completed breastfeeding more than 3 months prior, some had never been pregnant. The non-pregnant comparison group was asked to come to the laboratory on day 16-26 (luteal phase) of the menstrual cycle or were using hormone-based contraceptives.

Paper II

At the time of this paper, participants included in analysis were those that had finished the studies at the time of combining the data. This resulted in a total of 84 subjects for inclusion: 38 from UIC (the “urban” cohort) and 46 from UNC-Chapel Hill (the “suburban” cohort). The UNC cohort excluded individuals with a multiple gestation, non-English speaking, under 18, with past psychiatric history of bipolar disorder or psychosis by self-report and confirmed by the Structured Clinical Interview for DSM-5 (SCID-5_ (First et al., 2016), met criteria for a substance use disorder in the past 90 days and also confirmed by the SCID-5, and by self-report of history of major bowel surgery or having had inflammatory bowel disease (i.e., Crohn’s or Ulcerative Colitis). UIC excluded individuals non-English speaking, under 18, used medication besides prenatal vitamins, use of illicit drugs, use of tobacco, or had to have hormonal treatment for the current pregnancy (e.g., in vitro fertilization). The cohorts differed significantly by race/ethnicity, report of committed relationship, education level, age, and presence of obesity. In the urban cohort, individuals were excluded if they took antibiotics 6 months before the beginning of the study. Individuals in the suburban cohort were not excluded based on antibiotics, but a thorough medication history was taken at each visit (three at the first visit were taking an oral antibiotic, one at visit 2, five at visit 4; the individual who took an antibiotic in visit 2 also took one in visit 4 and was the only subject to take and antibiotic at more than one time point).
Paper III
Recruitment for the entire UNC cohort occurred from April 2017 through Au-
gust 2019. Subjects were recruited in the first or second trimester, with the
majority in the second trimester at intake. The UNC cohort excluded individ-
uals with a multiple gestation, non-English speaking, under 18, with past psy-
chiatric history of bipolar disorder or psychosis by self-report and confirmed
by the Structured Clinical Interview for DSM-5 (SCID-5) (First et al., 2016),
mets criteria for a substance use disorder in the past 90 days and also confirmed
by the SCID-5, and by self-report of history of major bowel surgery or having
had inflammatory bowel disease (i.e., Crohn’s or Ulcerative Colitis).
From 2016-2018, participants recruited at the routine ultrasound examina-
tion to be part of the BASIC cohort were invited to leave samples for analyses
of the microbiota (oral, vaginal and fecal samples), twice during pregnancy
and at 6 weeks postpartum.
For both cohorts, we did not exclude individuals in either cohort based on
medication usage, including antibiotic usage, but did assess if there were sig-
nificant differences based on presence of absence of medication. Bowel med-
ications included medications for Gastrointestinal Reflux Disease and medi-
cations for constipation.

Paper IV
BASIC included pregnant individuals that were followed up until 12 moths
postpartum. U-BIRTH included additional follow-up of participants and their
children at 18 months after childbirth. There were 2,616 mother-child pairs
that had at least two infant difficult temperament scores. 491 were excluded
as they did not participate in the 6-week postpartum questionnaire; leaving
2,125 pairs where temperament trajectories could be assessed. Another 438
were excluded due to missing data on covariates; leaving 1,687 for analyses.

Mental Health Assessments
Paper I
Subjects completed the Swedish versions of the EPDS and State-Trait Anxiety
Inventory for Adults (STAI) (Rubertsson et al., 2011; Spielberger et al., 1983).
The Life Incidence of Traumatic Events (LITE) was given to assess exposure
to childhood trauma (Greenwald & Rubin, 1999). Individuals attending the
laboratory visit were administered the Swedish version of the Mini-Intern-
tional Neuropsychiatric Interview (MINI) to determine presence of past or
current psychiatric diagnoses (Allgulander et al., 2009).
Paper II
The two cohorts share the Perceived Stress Scale-10 items (PSS-10) which has been found to have two factors including Emotional Distress and Self-Efficacy (Cohen et al., 1983; Taylor, 2015). The Patient Health Questionnaire-9 (PHQ-9) was collected in the urban cohort. The EPDS was used in the suburban cohort (Cox et al., 1987; Kroenke et al., 2001). The Generalized Anxiety Disorders-7 (GAD-7) was collected in both cohorts (Spitzer et al., 2006).

Paper III
The two cohorts share the EPDS; making it the tool utilized. The USA cohort utilized the SCID-5 for inclusion/exclusion criteria.

Paper IV
Maternal mental distress was assessed by the EPDS. Special attention was paid to the EPDS-3A, the three questions that assess anxiety (Matthey, 2008). Difficult infant temperament was defined from the Infant Characteristics Questionnaire (ICQ) (6 weeks), subscales of the Toddler Behavior Questionnaire (TBQ) (12 months) and the very short form of the Early Childhood Behavior Questionnaire (ECBQ) (18 months) (Bates et al., 1979; Bohlin et al., 1981; Hagekull, 1985; S. P. Putnam et al., 2006).

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24
Other Covariate Assessments

Paper I
BASIC gathered demographic and medical information from surveys as well as linkage to medical records (Axfors et al., 2019). At gestational week 17 and week 32, participants reported their age, country of birth, education, weight and height before pregnancy, employment status, usage of selective serotonin reuptake inhibitors (SSRI).

Paper II
Participants in the urban cohort completed demographic data through a survey and weight and height taken at the clinical visit. Participants in the suburban cohort completed demographic data through a secure web-survey with additional information asked at the research visit, weight and height taken at the research visit. Demographic data included age, self-identified race, whether the participant identified as Hispanic or Spanish-speaking, marital status, and education status. The presence or absence of gestational diabetes or preeclampsia was collected and reported.

Paper III
Participants in the USA cohort completed demographic data through a secure web-survey with additional information asked at the research visit. Weight and height taken at the research visit. BASIC gathered demographic and medical information from surveys; and gathered additional information through linkage to medical records (Axfors et al., 2019).

Paper IV
BASIC gathered demographic and medical information from surveys; along with linkage to medical records (Axfors et al., 2019). Relevant maternal sociodemographic data, collected at gestational week 17 and week 32, from the web-based questionnaires, included: age, country of birth, education, weight and height before pregnancy, employment status, history of depression, smoking history, usage of selective serotonin reuptake inhibitors (SSRI). Surveys in the postpartum week six included a question on the infant sex, partner mental health and whether there was additional support with the infant was available (e.g., partner support). Information about pregnancy length, birthweight, head circumference, parity, referral to the neonatal unit, and mode of delivery were gathered via linkage to medical records.
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Paper 1

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### Biomarkers

**Paper I**

Heart Rate Variability (HRV) was recorded using a Biotekna Photoplethysmography (PPG) with an electrode placed on the subject’s finger (Selvaraj et al., 2008). Two measurements of 5 minutes each were conducted before and after the Wechsler Digital Span Task (Wechsler, 1997). All time domain and frequency domain measures were calculated by the Biotekna device.

**Paper II**

**UIC/“Urban” Sample Collection**

Microbiome analysis from the urban cohort were almost all from rectal swabs (3 were from stool samples) which were processed and stored in a minus 80 Celsius freezer within two hours from collection in the clinic. Cytokine and chemokine analyses were from blood samples collected by trained phlebotomist during a clinical visit, processed into serum and then stored in the minus 80 Celsius freezer.
UNC/“Suburban” Sample Collection

Microbiome analysis from the suburban cohort were from stool samples. Participants were given a collection kit and instructed in home collection. Collection kit included a hat to put in the toilet, a flushable bag to collect the void, spatula and gloves to take sample and put in two tubes; one filled with RNA/DNA Shield and the other not containing any medium. Participants were instructed to put the tubes with samples into the cooler with the frozen ice pack. After the first visit, the participants notified the research team and the team member picked up the sample. For the other two visits, the research participant brought the sample in the cooler to the visit to give to the research team. The study team took samples to a laboratory hood and homogenized and aliquoted into tubes that were then stored in the minus 80 Celsius freezer. Cytokine and chemokine analyses were from blood samples collected by trained phlebotomist at the research visit, processed into sera and plasma, aliquoted, and frozen in minus 80 Celsius freezer. At time of analysis, UNC fecal and serum samples were shipped overnight with dry ice from Chapel Hill to Chicago.

Samples from both cohorts were extracted and barcoded at the University of Chicago, targeting the V4 region of the 16S rRNA gene. Collaborator Dr. Beatriz Peñalver Bernabé, was a fellow with Dr. Jack Gilbert’s laboratory at the University of Chicago and worked with the UIC Women’s Mental Health Program, carried out Amplicon Sequence Variants (ASV) with DADA2 (Callahan et al., 2016).

All serum samples from UIC and UNC were analyzed for the cytokine and chemokine analyses in duplicate at the University of Chicago using a T-cell specific panel from Milliplex.

Paper III

BASIC Sample Collection

Participants collected fecal samples at home and were instructed to sample from toilet paper with a spoon attached to the lid of the tube and put in the DNA/RNA shield (Zymo Research Corporation, CA, USA). Samples were mailed and stored at minus 80 Celsius freezer until DNA extraction.

UNC Sample Collection

Microbiome analysis from the USA cohort were from stool samples. Participants were given a collection kit and instructed in home collection. Collection kit includes a hat to put in the toilet, a flushable bag to collect the void, spatula and gloves to take sample and put in two tubes; one filled with RNA/DNA Shield and the other not containing any medium. Participants were instructed to put the tubes with samples into the cooler with the frozen ice pack. After the first visit, the participants notified the research team and the team member picked up the sample. For the other two visits, the research
participant brought the sample in the cooler to the visit to give to the research team. The study team took samples to a laboratory hood and homogenized and aliquoted into tubes that were then stored in the minus 80 Celsius freezer. At time of analysis, UNC fecal samples were shipped overnight with dry ice from Chapel Hill to the Karolinska Institutet in Stockholm, Sweden.

Microbiome sequencing was done at the Karolinska Institutet in Stockholm, all parent and child samples from both cohorts analyzed together. Samples were lysed with beads and DNA/RNA shield was used. Extraction and Whole Genome Sequencing data provided. Library preparation was carried out. Raw sequencing reads were trimmed to remove low-quality base pairs and adapters using fastp (S. Chen et al., 2018). Human reads were removed by k-mer based mapping to the Gch38 reference human genome with kraken2 (Wood & Salzberg, 2014). All sequencing data, depleted of human DNA, was deposited in the European Nucleotide Archive under project number PRJEB62678. Reads were taxonomically classified using Metaphlan3 (Beghini et al., 2020) and functionally annotated using humann3 (Beghini et al., 2020). Samples with less than 500,000 reads remaining after removing human reads were excluded from further analyses.

Paper IV

Biomarkers were not assessed in this study.

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29
Statistics

Paper I

Mean, range, standard deviations, and percentages of total BASIC third trimester participants and non-pregnant individuals with HRV measures were calculated for demographics, MINI diagnoses, and assessment scores. Missing data from the STAI and EPDS were imputed if only one response was missing.

New variables of HRV measurements were created to assess change from baseline to after the mental task by subtracting the baseline from the value after the task and dividing by the baseline.

Linear regressions were used to study the associations between HRV measurements and mental health measures: psychiatric diagnosis from the MINI, STAI score, EPDS score, the three subset questions of anxiety of the EPDS, and exposure to early trauma. HRV measures were plotted in histograms to assess normality. Two of the measures, Root Mean Square of Successive Differences between heart beats (RMSSD) and the low frequency band (LF) both were non-normally distributed so Mann-Whitney non-parametric test was used instead of linear regression. Fisher’s exact test was used to test BMI and age, important covariates, in relation to psychiatric diagnoses. Multiple testing was adjusted by applying a Bonferroni correction. P-values <0.00625 were considered significant. Statistics were done with the Statistical Package for the Social Sciences (SPSS) version 26.0 (IBM SPSS).

Paper II

Mean, range, standard deviations, and percentages of participants in each cohort separately were calculated for demographics, BMI, parity and whether obese. Chi-square tests were used for comparisons of categorical variables, t-tests for categorial and continuous variables, and Spearman partial and non-partial correlations for continuous variables. All were corrected with FDR.

A confirmatory factor analysis was used to explore how the two-latent variable perceived stress model fits for each cohort. As several questions did not factor the same way in the two cohorts, these questions had to be removed to create reduced perceived stress (rPS), reduced emotional distress (rED), and reduced self-efficacy (rSE). A ratio of rED to rSE was also created.
Alpha- and beta-diversity were calculated with phyloseq (McMurdie & Holmes, 2013). For alpha-diversity, we employed total observed amplicon sequence variance (ASV) and Chao to estimate richness; Shannon, Inverse Simpson and Abundance-based Coverage Estimator to estimate evenness. Beta-diversity distances were calculated with UNIFRAC (Lozupone & Knight, 2005) and based on samples rarefied above 6,000 counts. Statistically significant differences in alpha- and beta-diversity with respect to perceived stress dimensions (rED, rSE and rPS) were determined with PERMANOVA, adjusting by trimesters and cohort.

Associations between PS dimensions (rED, rSE, rPS) and ASVs were calculated using zero-inflated Generalized Linear Models adjusted by cohort and participant’s age; with statistically significant covariates determined by a stepwise approach. LMM for all reduced PSS-10 dimensions were corrected by statistically significant covariates that were determined using a stepwise approach. Normalized ASV counts were summarized at the genus levels to construct the taxa kinship matrix. Linear Mixed Models (LMM), following Rothschild et. al. (Rothschild et al., 2018), were used to identify the predictability of the Microbiome Associated Index with the total PSS-10 and the two factors, rED and rSE. For serum cytokines and chemokines abundance, models were corrected by participants’ BMI, age and cohort. Correlations between ASVs, the PS dimensions and cytokine/chemokine levels were analyzed to create co-abundance networks; significant edges of the network were three times greater than the standard deviations of the corresponding bootstrapping correlations and z-scores for the sample edge. Analysis was conducted in R (R Core Team, 2018) and figures were produced with ggplot2 (Wickham, 2009).

Paper III

All statistical analyses were done in R (R Core Team, 2015) version 4.2.2, graphical representations were created using ggplot2 (Wickham, 2009). For continuous, normally distributed variables, the Student’s t-test was used, for variables not normally distributed the Mann-Whitney U-test was used. For categorical variables the Chi-square test of independence was used. For all analyses, P < 0.05 was considered statistically significant. To adjust for multiple comparisons the Benjamini Hochberg Procedure was used.

The participants in each cohort were grouped in two groups based on their EPDS scores, those with EPDS>11 (higher distressed) or EPDS<=11 (lower distressed). Higher distress and the lower distress groups were compared in terms of demographic variables and scores on the EPDS. A confirmatory factor analysis was used to assess how EPDS questions group together by cohort and by time point.

Alpha diversity included: Richness (number of observed species), Pielou’s Evenness and Shannon-, Simpson’s-, Inverse Simpson’s Diversity indices.
The Mann Whitney U test ($\alpha = 0.05$) was used to compare the alpha diversity indices between higher distress and lower distress.

Models for differential abundance were created with EPDS higher and lower distress variables as the fixed effect and a random effect was added to correct for individual variation and/or BMI.

For beta-diversity, both principal coordinate analysis (PCoA) and distance-based redundancy analysis (dbRDA) were utilized to account for the variance in compositional nature of the sequencing data; and the account for variance in Gut Brain Modules (GBM). Gut Brain Modules (GBM) were used through reads that were taxonomically classified using Metaphlan3 and functionally annotated using humann3 (Beghini et al., 2020); converted to kegg and egg-NOG annotation (Darzi et al., 2016). Reactions of gut-brain modules which failed to be found were searched against the Uniprot database (The UniProt Consortium et al., 2023). A Robust Aitchison’s Distance framework was the basis for the analyses, chosen over Bray-Curtis as Aitchison’s Distance involves transforming the data with the robust centered log-ratio transform and then calculating Euclidean distances. The robust transformation uses observed features to estimate the geometric mean and 0’s are left in the data after the transformation. PCoA was done first and then dbRDA was used to see how constraining the data by EPDS distress and symptom types would result in variance between samples.

**Paper IV**

Infant factors such as sex, birth weight, head circumference and maternal factors such as age, BMI, and parity were analyzed with percentages of the total sample, median values, and interquartile ranges.

Infant temperament trajectories were calculated using the fussy difficult scale Infant Characteristics Questionnaire (ICQ) and the calculated difficult temperament subscales of the Toddler Behavior Questionnaire (TBQ) and Early Childhood Behaviors Questionnaire (ECBQ), using group-based trajectory modeling (Jones & Nagin, 2013; Nagin, 2005). Participants with more than one missing time point in the temperament scales were excluded. All scales were standardized to z-scores. The number of groups and the polynomial order were chosen based on the lowest Bayesian Information Criterium (BIC) and the Akaike Information Criteria (AIC).

EPDS questions were grouped into three questions for anxiety (Fawcett et al., 2019; Matthey, 2008), and the other questions were grouped and considered depression/anhedonia questions (K. T. Putnam et al., 2017). Correlations between temperament subscales and EPDS scores were assessed via Spearman’s Rho. Multinomial regression analyses were utilized to investigate associations between maternal depression/anhedonia and anxiety (anxiety measured via the EPDS-3A) and difficult infant temperament trajectories, while also accounting for other relevant maternal and infant factors/covariates.
Summary of Results

The first three papers study three types of biomarkers that provide additional information alongside mental characterization: 1) HRV, 2) microbial composition and functioning potential, and 3) immune factors. The fourth paper investigates maternal mental health in relation to infant temperament over 18 months; indicating how the characterization of maternal mental health, beginning in pregnancy, is important to both parent and child.

HRV and Mental Health in Late Pregnancy (Paper I)

Results of Paper I indicate HRV measures seem to reflect variation in autonomic nervous system function based on whether pregnant or not and based on specific anxiety types (i.e., obsessive compulsive versus exposure to greater trauma versus panic).

Non-pregnant individuals on average had higher values for HF RMSSD, LF and SDNN (7.2 +/- 1.3 vs. 5.7 +/- 1.3; 73.5 +/- 64.3 vs. 30.2 +/- 24; 83.1 +/- 47.8 vs. 53.5 +/- 22; 7.4 +/- 0.8 vs. 5.9 +/- 1.3).

Table IV shows the different mental health factors and significant findings where HRV values were different depending on the presence or absence of the mental health factor. Both presence of OCD and presence of exposure to five or more childhood traumatic events associated with lower HF measures (p=0.004, p=0.006); whereas presence of panic disorder, social phobia, trait anxiety from the STAI, and exposure to five or more childhood traumatic events all associated with LF to HF ratio (p=0.006, p=0.002, p=0.006, p=0.004). Exposure to greater trauma as a child was more likely to result in both lower vagal tone, but also an imbalance of low frequency (LF) band in relation to the high frequency (HF) band. The low frequency (LF) band does not necessarily only represent sympathetic tone and is thought to reflect the baroreflex maintaining balance of heart rate and blood pressure (Brugnera et al., 2019; Ernst, 2017). Panic varied by resting LF, whereas, social phobia varied by the resting ratio of LF to HF; panic and social phobia are both forms of anxiety, and yet, this study indicates they engage and associate with differences in ANS functions. HRV reflecting social phobia was more similar to the HRV reflecting exposure to trauma than HRV reflecting panic; as both social phobia and exposure to greater trauma associated with the ratio of LF to HF.
Trait anxiety related to higher LF/HF ratio after the stressor, possibly indicating these individuals with trait anxiety don’t have alterations at baseline, but have a harder time recovering from stressors. Change in VLF before and after the stressor was significantly different for those taking a SSRI; which could reflect the states underlying the need for SSRI or may reflect how SSRIs could be changing health. Depression was not found to be associated, but those identified as having a history or current depression may be a more heterogenous group.

**Table IV.** Significant differences in HRV values based on presence or absence of mental health factor

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<th>Panic Dis</th>
<th>Agoraphobia</th>
<th>Social Phobia</th>
<th>OCD</th>
<th>GAD</th>
<th>State Anxiety</th>
<th>Trauma Events</th>
<th>Fear of Childbirth</th>
<th>SSRI</th>
<th>Major Dep</th>
<th>Bipolar Mania</th>
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**Table IV.** Significant differences in HRV values based on presence or absence of mental health factor

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<th>Related to Stressor</th>
<th>Per cent Change</th>
<th><strong>Anxiety</strong></th>
<th><strong>Trauma Events</strong></th>
<th><strong>Fear of Childbirth</strong></th>
<th><strong>SSRI</strong></th>
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1Any fear of birth was not statistically significant. There were 43 individuals with any fear and 52 not reporting fear. Fear of childbirth included fear of C section, fear of vaginal delivery, and severe fear for any of the above. BMI was also included in the model for fear of childbirth only since it was only significantly associated with this variable.

2Age was included in the model for major depression since it was only significantly associated with major depression.

**Perceived Stress, the Microbiome, and Immune Factors in Pregnancy (Paper II)**

Paper II interrogates self-report of perceived stress in relation to characterization of microbial composition and T-cell related immune factors in two time points in pregnancy during the second trimester and third trimester. The study highlights the importance of determining if self-report items such as on the PSS are being answered in similar ways in demographically and geographically distinct populations. Several questions did not factor the same way in one population as in the other population. The factor analysis confirmed a model with two factors consistent with ED and SE. The items in each factor
that did not agree with the expected items in each factor included: item f from PSS-10 (“In the last month, how often have you found that you could not cope with all the things that you had to do?”); item e (“In the last month how often have your felt that things were going your way?”); and item h (“In the last month, how often have you felt that you were on top of things?”). To ensure consistency, reduced factors of SE, ED and for total PSS were used. Variance in SE was better explained when the Microbiome Associated Index was included in addition to demographic factors. Differential abundance analysis resulted in different patterns for taxa identified based on rSE, rED, and rPS. Network analysis noted significant connections between taxa, the PSS dimensions and T-cell related chemokines and cytokines. The most central node of the network was Bacteroides uniformis, which connected CXCL11 (ITAC) and Self-efficacy as seen in Figure I.

**Figure I.** Network analysis of taxa, perceived stress factor scores, and cytokine/chemokine levels

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**Mental Distress, Microbial Composition and Functioning in Pregnancy (Paper III)**

The mean EPDS scores differed more for USA participants between second and third trimester than for Swedish participants; the USA median EPDS scores significant differed between the two timepoints (p=0.016), but in the Swedish cohort, the two points did not significantly differ in EPDS scores (p=0.65). When applying a factor analysis, the Swedish cohort from both trimesters and the USA from the second trimester factored into questions 10 and
2, questions 3-5, questions 6-9, and question 10; USA in third trimester did not factor the same way.

Individuals reporting lower distress at both time points in both cohorts had significant differences in alpha-diversity across three different indices (Shannon entropy, Invert Simpson index, Pielou’s evenness) between second and third trimester time points (p=0.0049, 0.0029, 0.0096 for the three indices for Swedish individuals, p=0.023, 0.051, 0.015 for the three indices for the USA individuals); while alpha-diversity indices in second and third trimester of individuals reporting higher levels of distress at both time points were not significantly different. Microbial communities of Swedish individuals with higher distress were significantly different from individuals with lower distress (p=0.015); while only individuals with higher anxiety in the 3rd trimester USA microbial communities trended toward being different (p=0.076).

Differential abundance for individuals with higher distress included lower *Alistipes finegoldii* in both USA and Sweden. However, none of the taxa remained significant after consideration of multiple comparisons. Similarly, dbRDA plots of variance in microbial community composition constrained by mental distress identified some bacteria types such as *Akkermansia muciniphila* and *Ruminococcus bromii* appearing to be more contributory to the variance of microbial communities of individuals from the microbial communities of individuals with higher distress as characterized by the three subtypes; but these observations were not beyond random chance. These are taxa noted in the literature for associations with mental distress and have identified mechanisms that might explain their relationship to mental distress and improved mental health.

PCoA to assess variance in GBM potential functioning of microbial communities identified four distinct groups. When dbRDA was applied to the data, as seen in Figures II and Figures III, in order to constrain the data by mental distress, the groups identified were not beyond random chance. Functions contributing to variance were cortisol degradation, inositol synthesis, menaquinone synthesis, and short chain fatty acid synthesis, specifically acetate.
Figure II. dbRDA triplots showing the beta diversity at the two time points in the USA cohort. Dots are colored according to the four unified clusters found in PCoA plot in Figure S6a. The top 3 GBMs contributing the most to dbRDA1 and dbRDA2 (Cortisol degradation, Inositol synthesis and in Acetate synthesis III) were plotted with light blue vectors as described in Methods. The four EPDS subgroups were plotted with orange vectors according to their loadings. Four EPDS subgroups explained 2.9% of the variances in GBM composition among all samples with a non-significant p value from ANOVA test. EPDS_selfharm was more likely to drive the variation in GBM composition compared to the other three subgroups. EPDS_anhedonia and EPDS_anxiety also demonstrate a negative correlation in the USA cohort. ClusterOne and ClusterTwo are more abundant in the context of Cortisol degradation as well as Acetate synthesis III; while ClusterThree is more abundant in Inositol synthesis.
**Figure III.** dbRDA triplots showing the beta diversity at the two time points in the Swedish cohort. Dots are colored according to the four unified clusters found in PCoA plot in Figure S5a. The top 3 GBMs contributing the most to dbRDA1 and dbRDA2 (Cortisol degradation, Menaquinone synthesis I and in Acetate synthesis II) were plotted with light blue vectors as described in Methods. The four EPDS subgroups were plotted with orange vectors according to their loadings. Four EPDS subgroups explained 0.7% of the variances in GBM composition among all samples with a non-significant p value from ANOVA test. Both EPDS_selfharm and EPDS_anhedonia were found to be more likely to drive the variation in GBM composition comparing to EPDS_anxiety and EPDS_depression from the graph. EPDS_anhedonia and EPDS_anxiety demonstrate a negative correlation. ClusterOne and ClusterFour are more abundant in the context of Cortisol degradation; while ClusterTwo and Three are more abundant in Acetate synthesis II and Menaquinone synthesis I.

**Maternal Mental Distress and Infant Temperament Trajectories (Paper IV)**

Three trajectories of difficult infant temperament from six weeks to 18 months were identified as shown in **Figure IV**. Shown are means of estimated group trajectories at age six weeks, 12 and 18 months and associated predicted group percentages.

When adjusting for infant sex (boy/girl), maternal depression scores (EPDS depression/anhedonia) were significantly associated with higher odds of the
infant being in the stable medium trajectory (β=1.084, p=.027) while anxiety scores (EPDS-3A) during pregnancy were significantly associated with higher odds for the infant belonging to the high difficult temperament trajectory (B=1.273, p=.002).

**Figure IV.** Three trajectories identified of infants after birth to 18 months in relation to “difficult” temperament as defined by ICQ, TBQ, and ECBQ.
Discussion

The overall aim of this thesis was to study the integration of varied methods of defining perinatal mental health along with identification of biomarkers reflecting the microbiota-gut-brain axis and the ANS. Three key themes emerged from the four manuscripts included in this thesis: 1) the course of mental health in pregnancy is critical to the development of the parent and the child; 2) the characterization of perinatal mental health requires a mix of methods that recognize there may be differences in the use of methods based on the population; and 3) biomarkers of perinatal mental health need to reflect dynamic systems, and the components may not be as important as the patterns and interactions.

Theme 1: The course of mental health in pregnancy is critical the development of the parent and the child.

Paper I shows how late pregnancy is a “natural stress test” of the ANS (Bilhartz et al., 2011). The ANS and other aspects of the cardiovascular system are more activated in pregnancy to navigate to the needs of the pregnancy; but also still able to assess and react to situations deemed dangerous—these systems are critical to navigating pregnancy with balancing action when necessary, but not overreacting and not being excessive when not required. Paper I confirms prior work outside of pregnancy that HRV variations can reflect psychopathology (Beauchaine & Thayer, 2015; Shaffer & Ginsberg, 2017; Thayer et al., 2012). Furthermore, the study specifically noted differences in how psychopathology is reflected by HRV differently based on whether at rest or after a working memory task.

Paper II supports that the balance of emotional distress and self-efficacy is associated with different microbiome and immune factors. Combined with findings that perceived stress has been associated with the diversity of the microbial community and predicts later less emotional distress and greater self-efficacy postpartum (E. S. Long et al., 2023); the balance of self-efficacy and emotional distress in pregnancy is reflective of and important to how a number of systems are navigating pregnancy and setting navigation of perceived stress beyond pregnancy. Similarly, paper III indicates that individuals with lower distress in both 2nd and 3rd trimester may have more changes in systems reflected by the microbial community composition than individuals with higher distress in both time points. Pregnancy requires adaption and change of
numerous systems and mental health seems to impact how adaptable are systems. Greater self-efficacy and less distress enables adaptability not only in pregnancy, but ensures multiple systems developing in pregnancy critical to the developing parent are more adaptive and prepared for navigating parenting challenges.

Paper IV showed mental health of pregnancy is also important to the development of child’s temperament. Greater maternal anxiety in pregnancy is associated with a child having increasing challenges in navigating stress and for the parent supporting the child; challenges that seem to worsen across the first 18 months of life. Previous research with smaller sample sizes did not find associations between prenatal depression/anxiety and infant temperament (Nieto et al., 2019). Martini et. al., found an association between maternal prenatal anxiety and infant excessive crying and feeding problems (behavior which is also included in difficult temperament definitions e.g. ICQ), but no association between maternal prenatal depression and temperament (Martini et al., 2017). Our analysis found the association between anxiety in pregnancy and the high rising difficult infant temperament trajectory remained after adjusting for postpartum depression. Irwin et. al., have similarly found that increasing anxiety in pregnancy was associated with lower receptive language and gross motor skills of the children at 12 months than pregnant individuals with decreasing or stable-low anxiety (Irwin et al., 2020). Our results indicate that anxiety in pregnancy may be having impacts through prenatal programming. Depression/anhedonia in pregnancy was associated with the medium stable trajectory but got attenuated after introducing postpartum depression/anhedonia into the model. This may indicate there is something unique about anxiety in pregnancy in terms of programming.

Theme 2: The characterization of perinatal mental health requires a mix of methods that recognize there may be differences in the use of the methods based on the population.

These four papers support the use of a term such as PMADs that considers mental health in pregnancy involving not only depressive symptoms, but also different types of anxiety and greater exposure to childhood trauma. Furthermore, self-efficacy, in addition to emotional distress, is an important consideration, particularly in relation to the microbiome. Paper I utilized diagnoses from the MINI, but also showed the complex mix of co-morbidities that occur in using mental health disorder diagnoses. This work supports calls to use intermediate phenotypes beyond strict diagnostic criteria (Abi-Dargham et al., 2023).

The strength of the EPDS is that it is well validated and used world-wide and it is able to account for anxiety in addition to depression (Heller et al., 2022; Shrestha et al., 2016; Wickberg & Hwang, 1996). However, the studies also pointed to some considerations with the EPDS. Paper II showed the importance of using the EPDS more than at one time point. Trajectories of EPDS
scores between the two time points in paper II indicate several different groups (i.e., those with consistently lower distress, those with consistently higher distress, and those where distress varied between high and low in second and third trimester). EPDS scores also only reflect one method of characterization. For paper III, USA EPDS scores were lower than expected, especially given the high percent of participants with past history of major depression and/or anxiety disorders as determined by the study psychiatrist with clinical interview and the SCID-V (First et al., 2016). The use of the EPDS in the USA cohort did not always seem to match with clinical judgment of the study psychiatrist. Also of note, the urban cohort chose not to use the EPDS and instead used the PHQ-9 and GAD-7 as these are more commonly used as in non-perinatal primary care settings; and are still valid in pregnancy (Zhong et al., 2014).

Paper II and paper III created unique opportunities to study two different pregnant populations and to try to combine data; identifying places where there were similar findings and also identifying places where data could not be combined. Paper II showed that self-efficacy is just as important to consider as emotional distress. However, paper II also showed that several questions did not factor into self-efficacy and emotional distress the same way in the two populations. Similarly, paper III also found that the EPDS did not factor the same way in the two populations in the third trimester. Given the findings of paper II with the PSS-10, it may also be that EPDS may have regional differences within the USA in interpretation. Interestingly, the EPDS in the USA cohort in the second trimester factored the same way as the Swedish cohort, and what is more typical in the literature. This may indicate something unique about the third trimester compared to the second trimester for the USA cohort. The USA cohort was much smaller and so this may also contribute. While differences in sample size must be considered, both papers from three different populations indicate the importance of thinking about the components of mental health and being careful to not assume these components are the same in different populations. For Paper II, originally the team attempted to create our own factor analysis with the GAD-7, the PSS-10, and questions similar in the PHQ-9 and EPDS. However, in further discussion with the two teams it was decided it was better to utilize only the shared scale of the PSS-10 and the factors that have previously been shown in the literature (Taylor, 2015).

Paper IV also indicates that infant mental health can be characterized, but due to the development of the child multiple methods at different time points must be used.

Theme 3: Biomarkers of perinatal mental health need to reflect dynamic systems, and the components may not be as important as the patterns and interactions.

HRV measurements are an example that have promise as biomarkers, especially as HRV measurements are reflective of well-being and the health of
interactive systems; but should not be thought as reflecting not individual components of the ANS and cardiovascular system (Shiga et al., 2021; Von Rosenberg et al., 2017). Paper I indicates that because of high amounts of comorbidity, patterns of HRV measures compared between different aspects of mental health and both at rest and in relation to a task may be most helpful in reflecting perinatal mental health. By looking at patterns, we can identify similarities and differences between types of anxiety. For example, social phobia was more similar to greater exposure to trauma, both at resting state; while trait anxiety was more similar to panic in the type of measure that differed, but also panic and trait differed by the HRV response with regards to timing in relation to the working memory task.

Paper II indicates that biomarkers may be best identified and understood through network analysis; better understanding interactions of components. Network analysis narrowed down a large amount of data to identify Bacteroides uniformis as a key node in the network; strongly associating with higher distress, lower self-efficacy and also associates with CXCL11/ITAC. However, this also shows the tension in identifying biomarkers—the desire for one thing that is measurable and a marker of worse or better mental health. Bacteroides uniformis, inversely associated with SE and positively associated with rPS and the ratio of rED to rSE, has both been found in the literature to have positive and negative associations with health and inflammatory balance; and the complexity and importance of the interactions is further supported as B. uniformis connects Self-Efficacy to CXCL11/ITAC, an immune factor that is associated with the balance of immune suppression and activation (Agustí et al., 2021; Cole et al., 1998; Fabersani et al., 2021; Kulkarni et al., 2017; López-Almela et al., 2021; Y. Zhang, Fan, et al., 2022; Y. Zhang, Xu, et al., 2022; Zohar et al., 2014). The literature indicates another important factor in Bacteroides uniformis as a marker of whether beneficial or more pathogenic is whether it is in the presence of dietary fiber (Zafar & Saier, 2021); obese mice treated with a combination of Bacteroides and dietary fiber had curbed weight gain, improved glucose management, higher levels of the Short Chain Fatty Acid (SCFA) butyrate, and restoration of immune cells in the intestinal epithelium compared to untreated obese mice (López-Almela et al., 2021). The network captures how any one component is dependent on other parts of the system and cannot be considered in isolation as biomarkers. Higher CXCL11 has been identified in a panel of proteins of individuals with elevated depressive and/or anxiety symptoms both in third trimester and postpartum; but only identified when patients with preeclampsia were removed, and it is important to note, the smaller sample size for individuals without preeclampsia was 21 individuals (Accortt et al., 2023). Timing in pregnancy of the biomarker may also be important; different points in pregnancy require a different balance of immune tolerance and activation (Lokki et al., 2018). Specific biomarkers may only be effective in certain populations (e.g., with or without obstetric complications) and in concert with other factors such as dietary fiber.
consumption. This all suggests that biomarkers must be considered in terms of the context of the timing in pregnancy and the population of interest.

Paper III questions whether specific taxa of importance can be identified. It is tempting to think of specific types of taxa as biomarkers given things that can be found in the literature and the desire for interventions such as probiotics (i.e., giving specific types of bacteria to promote health). *Alistipes finegoldii* was identified in both cohorts, but does not remain significant after correction for multiple comparisons are applied. Similar to *Bacteroides uniformis*, *Alistipes finegoldii* has been previously found to both be pathogenic and protective (Parker et al., 2020). *Alistipes finegoldii* has been associated with a number of functions that may be important in pregnancy and in relation to greater mental distress; functions that could be necessary and protective at certain times while pathogenic at other times such as metabolizing tryptophan to indoles, shifting of immune cells from T regulatory cells to Th17 cells, and associations with increased inflammation (Jama et al., 2019; H. Jiang et al., 2015; Kim et al., 2018; Parker et al., 2020). *Ruminococcus bromii*, *Akkermansia muciniphila*, and *Roseburia* species were identified from the USA sample to appear to be associated with less of the three subtypes of distress, but, again, are not beyond random chance. All three have been noted in the literature previously with regards to mental distress (Bao et al., 2021; Becken et al., 2021; Cani et al., 2022; Chang et al., 2023; T. Chen et al., 2021; Dao et al., 2016; David et al., 2014; Derrien et al., 2011; Ding et al., 2021; Kim et al., 2021; McGaughey et al., 2019; Nie et al., 2021; Sasaki et al., 2022; Shen et al., 2022; Zhai et al., 2019; T. Zhang et al., 2019). As *Ruminococcus bromii* and *Roseburia* spp. have been identified as highly dependent on plant versus animal-based diets, one could hypothesize about how these were reflected differently in the two population as the USA and Swedish likely differ in dietary practices, and even within the USA there is likely variation in the Chapel Hill cohort in dietary practices among participants (David et al., 2014). The literature repeatedly identifies taxa of interest and yet results are often not replicable. Given the community nature of the microbiome, the individual taxa likely are highly impacted by the communities, the other members of the communities, in which they reside.

Paper III findings suggest that functioning of the community may be a more important direction of study in order to identify biomarkers that could reflect how pregnancy is being navigated; with a look towards improved mental health of parent and child going forward. Functions contributing to variance among microbial communities were cortisol degradation, inositol synthesis, menaquinone synthesis and short chain fatty acid synthesis, specifically acetate. Short chain fatty acid (SCFA) production is often cited as an important function of the microbiome in pregnancy, impacting the host immune system, and as relates to mental health (S. Chen et al., 2023; Corrêa-Oliveira et al., 2016; Sanna et al., 2019; Silva et al., 2020). The SCFA acetate has been found
when used as supplementation in an animal model of chronic social failure stress to improve depression-like behaviors (W. Huang et al., 2021). However, in a study of 164 individuals (125 with psychiatric conditions), acetate was higher in individuals with higher depression symptoms and higher amounts of diarrhea (Müller et al., 2021). This also suggests that metabolites must be considered in relation to other components of the systems in which they are created. Functions in isolation will not be effective as biomarkers of mental health without consideration of context and interactions of different components. The identification of cortisol degradation may support a possible mechanism for transgenerational transmission via the microbiome of stress regulation, as the microbiome has been implicated in the development of the HPA axis (Sudo et al., 2004). Depression in pregnancy has been associated with higher newborn hair cortisol (Karl et al., 2023). Menaquinone, or vitamin K, synthesis was identified in the Swedish cohort; and has been found in an animal model to reduce social anxiety, reduce depressive behavior, and enhance memory performance (Elkattawy et al., 2022). However, menaquinone synthesis in microbial community functioning has also been associated with sleep problems (Pedroso et al., 2022). Inositol synthesis was found in the USA cohort; and lower myo-inositol is associated with depression and sleep problems (Ghosh et al., 2022; Pedroso et al., 2022; Siracusa et al., 2022; Urrila et al., 2017). These latter two differed by cohort and indicates the challenge of finding biomarkers across populations and may require identification of biomarkers specific to specific populations.

Papers II, III, and IV indicate the important of longitudinal data; and in particular, the trajectories of paper IV indicate that constructs such as temperament are not always stable, particularly for temperaments reflecting more sensitivity to change. In order to identify biomarkers, processes must be characterized over time.
Methodological Considerations and Limitations

The sample sizes are some of the largest longitudinal studies to investigate the microbiota-gut-brain axis and HRV in pregnancy, but this work indicates even larger cohorts and more timepoints are needed to increase accuracy of findings; especially during the dynamic perinatal period, and given the number of covariates creating high likelihood of findings that are not beyond random chance. Longitudinal data provide the opportunity to assess trajectories, but also limit analysis of certain data points if they were not available at the different time points. It is also difficult to ensure collection of self-report tools always matches with collection of samples.

Paper II and paper III created a unique opportunity to study three different pregnant populations and to try to combine data; however, this also led to limitations. Paper II allowed the samples from the two USA cohorts to be combined for a larger samples size; but this limited analysis to the PSS-10, as this was shared between the two. In the case of paper III, it was determined it was better not to combine the data. If the two cohorts in paper III were similar in size as in paper II, it might have been easier to combine data. However, it would have still been important to do the factor analyses of the self-report tools. Considerations for contribution to differences in response to the self-report tools may be ways participants were recruited and in what setting the tools were given (i.e., in a clinic visit versus in a laboratory visit, flyers for recruitment as opposed to being asked during clinical visit); one could hypothesize a clinic setting may lead to participants feeling less like they can disclose distress for fear of being judged by their treatment team and worries of being determined unfit to parent; whereas a participant who chooses to be in research outside of clinical care may feel okay to disclose distress as that is a point of the study. Participants may worry that the psychiatrist giving the tools, even in a research setting, may try to provide medication for higher scores of distress and they may not want medication. For Paper III, it is difficult to ascertain if the USA cohort is fundamentally different in answering and utilizing the EPDS from the Swedish cohort, or if the smaller sample size contributed. Paper II required taking out questions which may have led to loss of important components of the PSS. In order to overcome issues with reproducibility between laboratories, a strength of both papers is that samples in each paper from different cohorts were analyzed together by the same laboratory at the same
time. By presenting both cohorts in paper II, it still allows for contrast and comparison.

Decisions around choice of mental distress tool, can have significant impacts on assessing biomarkers. The first set of analyses done of the combined USA cohorts used a factor analysis of the PHQ-9, EPDS, GAD-7 and PSS-10 combined. When utilizing these factors with the cytokine data, IL-23 was found to be significantly different in relation to anxiety (Bernabe et al., 2020). A master’s student was able to replicate IL-23 in relation to anxiety measured from the GAD-7 in the entire USA UNC cohort. IL-23 has been associated with Th17 cell differentiation; Th17 cells may be another important mechanism, beyond ITAC, in immune tolerance versus inflammation as Th17 cells protect against external microbes abut in excess leads to excessive inflammation (Revu et al., 2018; Slyepchenko et al., 2016). This identifies how the choice of tool may identify different biomarkers of interest.

Even with three different populations from which participants were recruited, these are still not reflecting the diversity of pregnant individuals. There are patient populations in the three cohorts that are not included. For example, for Paper III, the Swedish and the USA cohorts are similar in that both are recruited from communities highly connected to universities; reflected by both cohorts had very high average education levels. Individuals willing to participate may have their own ties to academic research whereas individuals who chose not to participate may have had negative interactions with research. Patient involvement in design of studies and greater outreach might increase diversity of participation so that research better reflects clinical populations.

There are methodological considerations in relation to measurement of HRV. Paper I utilized photoplethysmography (PPG), which is more easily captured and has the potential for use in a number of settings including the patient’s home, but EKG is the gold standard for HRV research. Since data was analyzed by the Biotekna machine, we are unable to comment on methods of accounting for noise. EKG raw data allows the research team to make data processing decisions, including decisions around controlling for noise.

There are methodological considerations in relation to microbiome analysis. Microbiome analysis requires decisions made all along each step of the pipeline that may impact the results. Results are dependent on the reference libraries chosen. For Paper II, originally data was analyzed with QIIME1 and then reanalyzed for this paper with QIIME2. For paper III, an Aitchinson framework was chosen over Bray Curtis, creating differences in how taxa with zero relative abundance are included in the analyses. Similarly, the GBM are limited by what is already known about different genes and the libraries that associate genes with functions. Another consideration is the lack of dietary data. Both USA cohorts had dietary diet, but the data was not consistent between the two. Each dietary capture method has limitations such as recall bias and a balance between too much data or over-simplification of diet.
There are methodological considerations with regards to characterization of infant mental health. Paper IV was limited to parent report and lacks objective measures of infant stress reactivity such as cortisol or HRV in relation to a stressor. The three tools utilized each accounted for development of the child but assessed “difficult” temperament in different ways; in the trajectories these had to be assumed to be similar enough. Studies of parent and child together require larger sample sizes to account for the many data points.
Most important findings

- Different types of past or current anxiety disorders, greater trait anxiety, and greater exposure to childhood traumatic events were reflected by HRV profiles, at baseline and in relation to a mental task stressor, indicating different autonomic nervous system contributions.
- The gut microbiota data improved the prediction of self-efficacy levels compared with models based on socio-demographic characteristics alone.
- A network analysis approach identified the possible mediation effect of *Bacteroides uniformis*, bacteria found in the literature to be more beneficial in the presence of dietary fiber, between an immune factor, important in immune tolerance, and self-efficacy.
- The PSS-10 and the EPDS did not factor the same way when comparing the cohorts.
- Characterizing microbial communities of individuals based on potential functioning led to identification of variance between communities due to cortisol degradation, inositol synthesis, menaquinone synthesis and short chain fatty acid synthesis, specifically acetate.
- Maternal anxiety in pregnancy was associated with children who had higher levels of sensitivity and with greater negative affectivity that continued to increase over early life.
- In order to study different cohorts, it is important to ensure tools mean the same thing in the different cohorts, assays are done together when possible, and that sample sizes are comparable if combining data.
Clinical Implications and Future Perspectives

Just as the patient described at the beginning has an intricate story that develops over time, the results of these four papers reflect the complex and dynamic interplay of characterizing mental health in pregnancy. The results of these papers indicate the importance of thinking of biomarkers within context and in relation to interactions and patterns of a number of components of mental health. Larger sample sizes will be required to further subtype individuals based on trajectories of symptoms, different types of symptoms and symptoms along dimensions of level of distress, contributions such as past history of depression and anxiety and the presence of co-morbidities. Parent and child must be thought of in terms of their interactions and impacts on one another beginning in pregnancy. This work has shown it is possible to combine data from populations with different demographics and from different geographies; and it is necessary to do so as it provides a more generalizable results beyond one population. However, this work has identified some important considerations around standardizing use of tools and specimen collection, analyzing samples together when possible, and recognizing self-report tools may not be universally interpreted the same way across different populations. Future work will need to continue to rely on current collaborations and develop new collaborative efforts; and patients need to be included as partners in these collaborative research efforts (Abi-Dargham et al., 2023; Kirkpatrick et al., 2021).

Much of the research to date has been through laboratory studies; and while study in the laboratory makes it easier to overcome signal quality issues it also creates limitations in terms of applicability to use in clinical care (Arakaki et al., 2023). HRV serves as an example of the challenges in being able to utilize HRV as a biomarker of mental health. There is progress in use of tools such as wearables and the tools for decreasing noise in the data, improved computational ability to capture and analyze more nuanced data across time and in relation to stressors for individuals; capitalizing on this progress is necessary to overcome challenges in the use of HRV measurements as biomarkers of mental health, moving beyond a general marker of poorer health, increasing reliability and being able to inform decision-making with respect to specific interventions and the timing of specific interventions (Abi-Dargham et al., 2023; Arakaki et al., 2023). There is limited study of HRV biofeedback, that is utilizing monitoring of HRV by the individual to improve their health; but a meta-analysis of 14 randomized controlled studies indicates that in most of
the studies HRV biofeedback significantly improved depressive symptoms (Pizzoli et al., 2021).

HRV and the microbiome may be improved as biomarkers if they can be studied and utilized simultaneously. Combining HRV measures with measures of the microbiome may better reflect the anti-inflammatory cholinergic reflex and HPA axis activity (Bonaz et al., 2018; Harris, 1950; Pavlov et al., 2003; Ravi et al., 2022; Tracey, 2002). Future work should continue to assess maternal anxiety in pregnancy as some anxiety is necessary to navigate a dynamic period, and we do not yet know when anxiety is excessive. It is important to study maternal anxiety in relation to maternal and infant outcomes to better assess when anxiety requires intervention. Inclusion of HRV and the microbiome has the potential to better understand maternal anxiety in relation to the parent’s and the child’s developing and adapting anti-inflammatory cholinergic reflex and HPA axis. Future work should consider interrogating microbial functioning such as microbiome cortisol degradation and HRV biomarkers of both parent and child at rest and in response to stressors. Future work would benefit from biomarkers that are identified for both parent and infant, starting in utero and continuing through the first 18 months after delivery.
Conclusions

While these four papers shed light on only a small part of a complex larger dynamic system, they highlight three key themes: 1) the course of mental health in pregnancy is critical to the development of the parent and the child; 2) the characterization of perinatal mental health requires a mix of methods that recognize there may be differences in the use of the methods based on the population; and 3) biomarkers of perinatal mental health need to reflect dynamic systems, and the components may not be as important as the patterns and interactions. HRV patterns vary depending on type of anxiety and mental health traits such as SSRI use; all indicating different autonomic nervous system and cardiovascular contributions being contributory. The microbiome differs in relation to self-efficacy versus emotional distress; self-efficacy, microbiome, and immune factors suggest a complex network that may have the potential to reflect immune tolerance and activation. Microbial potential functioning is another possible way to characterize individuals; and the microbiome indicates functions including acetate synthesis, cortisol degradation, inositol and menaquinone synthesis are important functions for future study. Anxiety in pregnancy deserves greater attention as it associates with children that are more sensitive and less stable over time in their temperament. This work supports that pregnancy is a critical time for the mental health of both parent and offspring; and that the microbiota-gut-brain axis and HRV hold promise in terms of biomarkers reflecting mental health and pointing to new ways to develop more precise care plans improving mental health outcomes for parent and child.
Sammanfattning på svenska

Perinatala humör och ångeststörningar

Perinatala humör- och ångeststörningar (PMAD) är vanliga och har långvariga effekter på förälder och barn som sträcker sig bortom graviditet och postpartum. Karakterisering av mental hälsa under graviditeten komplicerar av graviditets dynamiska natur som inkluderar biologiska, sociala och psykologiska förändringar. Syftet med denna avhandling var att studera system som kan återspeglasa och integrera mått på fysiologiska komponenter och personens karakterisering av sin mentala hälsa. Detta arbete fokuserade på flera metoder för karakterisering av mental hälsa och tre typer av biologiska system, inklusive det autonoma nervsystemet, vilket återspeglas av hjärtfrekvensvariabilitet, immunsystemet och mikrobiota-tarm-hjärnaxeln som återspeglas i mikrobiomsammansättning och potentiella mikrobiella gemenskap fungerar.

Biomarkörer

Biomarkörer försöker vara indikatorer på medicinska tillstånd eller processer. Patientberättelser om deras psykiska hälsa innehåller ofta beskrivningar av fysiska symtom som hjärtslag. Hjärtfrekvensvariabilitet (HRV) tros återspeglasa olika komponenter i det autonoma nervsystemet (ANS); med större variation som anses speglas ökad anpassningsförmåga och förbättrad hälsa. HRV kanske kan speglas hur en individ navigerar förändringar. Forskningen är dock begränsad med avseende på bredden av karakteriseringar av mental hälsa, och i synnerhet med avseende på PMAD. Graviditet innebär många förändringar av immunförsvarvaret. På liknande sätt leder mikrobiomet både till förändringar och svarar på förändringar i immunsystemet och kan vara en markör för integrationen av ett antal viktiga system för hälsan.

Maternell stress och spädbarnsresultat

Maternell stress är inte bara förknippad med negativa resultat för den gravida personen, utan också för avkomman. Transgenerationell överföring av stress som kan återspeglas i förändringar i barnets reaktivitet, självreglering och interaktion med omgivningen och andra individer.
Mål

Det övergripande syftet med denna avhandling är att studera sambanden mellan olika metoder för att karakterisera perinatal mental hälsa med biomarkörer som speglar ANS och mikrobiota-tarm-hjärna-axeln.

I. Att analysera HRV-profiler under sen graviditet före och efter en mental uppgift i relation till psykiatriska diagnoser och psykiska hälsosfaktorer

II. För att bestämma hur upplevd stress och dess komponenter av nöd och själveffektivitet under graviditeten i två olika USA-kohorter (en förorts-, hög-resursrik och den andra urbana, mindre resursrika) är associerade med mikrobiell sammansättning och immunfaktorer

III. Att använda både en USA-kohort och en svensk kohort, för att utforska självrapporterade mentala nödmått i relation till mikrobiell sammansättning och mikrobiell potentialfunktion

IV. Att identifiera distinkta banor av svårt spädbarns temperament, som rapporterats av modern under de första 18 månaderna efter förlossningen; och att bedöma banor i relation till moderns symtom på depression och ångest under graviditet och postpartum

Metoder


Resultat

PSS-10 och EPDS påverkade inte på samma sätt när man jämförde kohorter. Olika typer av tidigare eller nuvarande ångeststörningar, större karaktärsängest och större exponering för traumatiska händelser i barndomen återspeglades.
av HRV-mönster, vid baslinjen och i relation till en stressfaktor av mental uppgift. Data från tarmmicrobiota förbättrade förutsägelser av själveffektivitetsnivåer. Ett tillvägagångssätt för nätverksanalys identifierade den möjliga medieringseffekten av Bacteroides uniformis, bakterier som är mer fördelaktiga i närvaro av kostfiber, mellan en immunfaktor, viktig för immuntolerans, och själveffektivitet. Karakterisering av mikrobiella samhällen av individer baserat på potentiell funktion ledde till identifiering av varians på grund av kortisolnedbrytning, inositolsyntes, menakinonsyntes och kortkedjig fettysyrasyntes, särskilt acetat. Maternell ångest under graviditeten var förknippad med barn som hade högre nivåer av känslighet och med större negativ affektivitet som fortsatte att öka under tidigt liv.

Slutsatser

Även om dessa fyra artiklar belyser endast en liten del av ett komplext större dynamiskt system, belyser de tre nyckelteman: 1) förloppet av mental hälsa under graviditeten är avgörande för utvecklingen av föräldren och barnet; 2) karakteriseringen av perinatal mental hälsa kräver en blandning av metoder som inser att det kan finnas skillnader i användningen av metoderna baserat på befolkningen; och 3) biomarkörer för perinatal mental hälsa behöver spegla dynamiska system, och komponenterna kanske inte är lika viktiga som mönstren och interactionerna.
Acknowledgements

This work was carried out at the Department of Women’s and Children’s Health, Uppsala University; with additional support from the Department of Medical Sciences, Psychiatry, Uppsala University. This work would also not be possible without collaborations with the University of Illinois at Chicago, the Karolinska Institutet Stockholm, and the University Hospital of Tübingen, Germany. My research has been made possible through the support of the University of North Carolina at Chapel Hill.

Alkistis Skalkidou, my main supervisor, for being a role model in carrying out the research that will improve perinatal health not only for the pregnant person, but for the whole family. The BASIC cohort continues to inspire me and gives us the opportunity to better understand many facets of perinatal mental health. Thank you for all your support, both in the specific research presented in this thesis, but also in the pursuit of science as a clinician.

Janet Cunningham, my co-supervisor, for being a role model in four generation problems; for melding together clinical knowledge of psychiatry with striving to better understand the integration of biological underpinnings. None of this would be possible if we had not had so much fun talking science while exploring Venice. And, also, to your boys for always making me feel at home when I was away from my boys.

Emma Fransson, my co-supervisor, for being a role model in systematic science that never loses sight of humanity. Your time and dedication working with me on the different analyses, reminded me I could be a scientist and continues to encourage me to keep going at being a clinician scientist. I greatly appreciate all our conversations that span across so many topics (even Swedish children’s literature).

Samantha Meltzer-Brody, my mentor at UNC and co-supervisor for this work, for being a role model in leadership in perinatal mental health, and psychiatry, in general. None of this would be possible had you not recruited me to UNC and you had not encouraged and supported me in the development of my K23 award project.
Luisa Hugerth, Bangzhuo Tong (Ben), and Alfons Edborn Devall for teaching me so much about microbiome analysis and continuing to improve on what we can learn about the microbiota-gut-brain axis in the perinatal period.

Ferdinand Sörensen for being my collaborator in learning more about infant temperament and for allowing me to bring my interest in perinatal anxiety into the project.

Beatriz Peñalver Bernabé for being my collaborator throughout my K23. I knew from the first time we met at the perinatal mental health meeting in Chicago that I had found a kindred spirit. Thank you for having the same passion for science, and particularly, the microbiota-gut-brain axis and perinatal mental health. Please also thank the entire UIC Women’s Mental Health Research Program for making our collaboration together possible. I have learned such a great deal about characterizing perinatal mental health and am inspired in how the UIC team shows what is possible in women’s mental health research.

Allison Eriksson and Richelle Björvang for great conversations and for being able to support one another in our work. Allison, your boys understand my boys love of ketchup.

Tamar Gur for also being a kindred spirit, encouraging me to keep on, in the pursuit of knowledge of the microbiota-gut-brain axis in order to improve the care we provide to pregnant individuals and their children.

Jennifer Payne, Barbara Schindler, Owen Montgomery, and Ana Núñez for being important mentors in women’s health earlier in my medical career.

The North Carolina Maternal Mental Health MATTERS (NC MATTERS) team who are doing the work of ensuring better access to perinatal mental health. Together we have continued to learn about perinatal mental health, the ways to assess for it, the ways to characterize and improve it.

Hannah Rackers, Jamie Steed, Shirin Ataei Kachouei, the group of UNC undergraduates, and the participants who made the collection of the data from the K23 cohort possible.

Marcé of North America, International Marcé Society, Society of Biological Psychiatry, the American College of Neuropsychopharmacology, RiseUp-PPD, Microbiome Movement: Maternal & Infant Health, Mind Mood & Microbes, the European Behavioral Pharmacology Society workshop on the microbiota-gut-brain axis, and the Bordeaux Summer School for giving me opportunities to share my work and to continue to refine what we can learn from the data.
This work would not be possible without the funding support of a NIMH training grant 1 K23 MH110660-01, a Brain & Behavior Research Foundation NARSAD Young Investigator Award, the P&S Fund, the Arnold O. Beckman Postdoctoral Award, the K-12 BIRCWH Award (K12HD101373) to Beatriz Peñalver Bernabé, the Swedish Research Council (Grant numbers 523-2014-2342, 523-2014-07605 and 521-2013-2339), the Göran Gustafsson Foundation, the Swedish Brain Foundation, Marianne and Marcus Wallenberg Foundation, the Söderström Königska Foundation, the Swedish Society of Medicine, the Crime Victim Compensation and Support Authority, the P.O. Zetterling Foundation, the Swedish Council for Working Life and Social Research, the Family Planning Foundation, the Fredrik and Ingrid Thuring Foundation, the Födelsefonden, The General Maternity Hospital Foundation; the Gillbergska Foundation, the Mårta and Nicke Nasvell Foundation, the Professor Bror Gadelius Memorial Fund, and the Naeslund Scholarship, the SciLifeLab & Wallenberg Data Driven Life Science Program (grant: KAW 2020.0239). Sample collection of the BASIC cohort and DNA sequencing were made possible by support from Ferring Pharmaceuticals.

My parents and brother for helping me become me. My mom, with whom I can laugh and have great conversations, gives me a love of art and learning. My dad, who has taught me quiet thoughtful strength, gives me the confidence to continue in the pursuit of knowledge and that every challenge is “another opportunity to excel.” My brother, with whom is my favorite person to discuss literature, gives me laughter and the knowledge someone is always in my corner.

My boys (my husband, Ryan, and my sons, Jack and Andrew) who inspire me daily to be a better human. There is too much to say. I cannot begin to express the importance of your love and support; and how much I appreciate that you get how important this work is to me.


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## Appendix

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<th>Questionnaires (not included in papers)</th>
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